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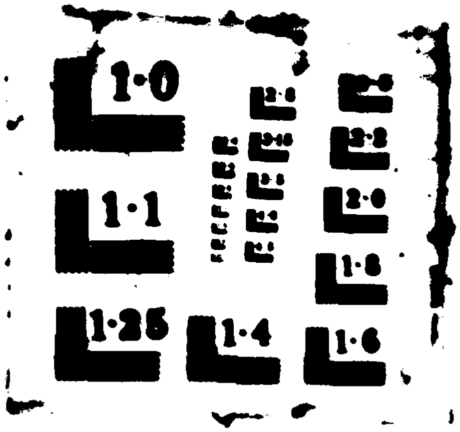
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IMPERMEANT SOLUTES AND CELLULAR CALCIUM METABOLISM IN PATHOGENESIS OF ACUTE RENAL FAILURE

Annual Report

Robert W. Schrier, M.D.

February 1985

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Using a rat model of in vivo renal ischemia and one of gentamicin-induced nephrotoxicity as well as the freshly isolated preparation of rat proximal tubules, it was found that adenosine triphosphate levels of rat kidney cortex improves for 3-6 hours after anoxia but then subsequently declines with continued reperfusion; that calcium accumulates in mitochondria in vivo during reflow; that in vitro mitochondria return to normal buffering capacity soon after reflow begins but thereafter this capacity is lost. Finally, endoplasmic reticulum are better preserved as calcium transporting vessicles than are mitochondria over

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and gentamicin in vivo induces early, and increases in an intracellular calcium compartment or pool even before functional and morphologic injury is seen.

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SUMMARY

In the second year of this contract, we examined the effect of, in the rat, reflow induced cytosolic and mitochondrial calcium overload. Based on our hypothesis that a large portion of this accumulation of calcium in mitochondria had already occurred in vivo prior to harvesting the mitochondria, we added ruthenium red to the isolation medium. In this procedure, calcium uptake and release by the mitochondria would be minimized. We found that indeed, at least 50% of the calcium seen after harvesting mitochondria was found to have already occurred prior to isolation. We also measured calcium buffering by these same mitochondria and measured the adenine nucleotide content of renal cortex during reperfusion after ischemia. Our studies of endoplasmic reticulum demonstrated that these calcium transporting membranes were intact after ischemia. Mitochondrial injury was therefore rather unique. We also examined gentamicin-induced renal injury and determined that toxic renal injury like ischemia is preceded by a gradual, progressive increase in intracellular calcium content. Finally, we began to study if verapamil or nifedipine could prevent anoxia-induced calcium influx in freshly isolated tubules. These studies were designed to evaluate cell injury and protection in the absence of changing vascular factors.

FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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BODY OF REPORT

After our observation that calcium accumulates in both dog and rat mitochondria during reflow, it became apparent that some or all of this overload might have been due to technical artifacts such as lysis of cortical tissue which was calcified after which the calcium in the homogenate might enter the mitochondria during mitochondrial enrichment. Our studies using ruthenium red demonstrated that this artifact did occur but that at least 50% of the calcium seen in mitochondria had already been accumulated prior to harvesting the cortical tissue.

In further studies, adenosine triphosphate levels were noted to increase toward normal during reflow but that in parallel with the subsequent deterioration of mitochondrial respiration between 6 and 24 hours, adenosine triphosphate levels also declined. Calcium uptake and release by mitochondria, an in vitro measure of the buffering ability of mitochondria followed the same pattern, that is improving by 3 hours of reflow and declining thereafter.

The unique injury to mitochondria with reflow was not paralleled by any such injury to the endoplasmic reticulum which could take up calcium in a normal fashion at both one and 24 hours after a 50 minute ischemic insult.

We have initiated studies of the role of changing phosphate on the severity of renal ischemic mitochondrial respiratory damage, calcium overload and adenine nucleotide homeostasis. We have also made progress in evaluating the effects of gentamicin on cellular calcium handling by isolating proximal tubules from rats given gentamicin for either one or 3 days. These studies are intriguing since preliminary data suggest that even before morphologic or functional injury, there is induced a progressive increase in intracellular calcium compartment size. This observation suggests that possibly gentamicin, like ischemia, can cause renal injury via progressive calcium overload. In gentamicin-induced injury this may take several days to reach a level, at which further membrane and cellular injury seen as necrosis, that acute renal failure would become overt.

Conclusion

These studies as well as future studies, specifically planned to examine calcium injury in cultured cells and calcium flux studies on freshly isolated tubules with gentamicin- or anoxic-induced acute renal failure, are directed at evaluating the role of calcium in cell injury often in the absence of a changing vascular response after insult. It is clear now that ischemia itself renders mitochondria but not endoplasmic reticulum incapable of buffering any increase in cytosolic calcium. With reflow much calcium enters renal tubules and transiently this is buffered by mitochondrial uptake. A point is reached, however, at which mitochondria become injured by the progressive increase in calcium and their recovery process is abruptly terminated. Therefore, between 6 and 24 hours of reflow adenosine triphosphate synthesis falls, calcium overload progresses and cells become necrotic. Gentamicin may injure cells through a similar calcium dependent mechanism but one which is somewhat slower. Three papers have been submitted on this work (1,2,3).

Recommendation

It is becoming clear that judicious use of agents or maneuvers to prevent calcium overload in vivo can prevent ischemic cell injury. These studies

should be extended to the gentamicin model and be performed on more isolated cell systems in which vascular (in vivo) responses to the maneuver do not make data interpretation more difficult.

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